

The influence of dexamethasone and the role of some antioxidant vitamins in the pathogenesis of experimental bronchial asthma

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Background: Bronchial asthma is a disease characterized by paroxysmal and reversible obstruction of the airways. The imbalance between the oxidant and antioxidant system that is called oxidative stress is critical in asthma pathogenesis. It is likely, therefore, that antioxidants may be effective in the treatment of asthma. Systemic treatment with glucocorticoids has been reported to inhibit smooth muscle hypercontraction which may account partially for their beneficial effects in the treatment of asthma.

Objective: The present study was conducted in order to study the effect of dexamethasone and some antioxidant vitamins on interleukin-4 (IL-4), immunoglobulin E (IgE) and heat shock protein 70 (Hsp70) in bronchial asthma in rats, and to recognize their possible beneficial role.

Method: The study was conducted on 60 adult male albino rats randomly divided into 4 groups (15 for each group): including normal control group (group A); asthma model group where rats were sensitized by ovalbumin and challenged with antigen aerosol producing bronchial asthma (group B); asthma model group treated with antioxidant vitamins (vitamin E and vitamin C) (group C); asthma model group treated with dexamethasone (group D). Blood and lung samples were collected from all groups.

Results and Conclusion: Our results revealed a significant decrease of serum reduced glutathione (GSH) levels among groups B, C and D as compared to group A, while there was a significant increase in group C and D as compared to group B. Antioxidant and dexamethasone treatment resulted in a significant decrease of serum IL-4, malondialdehyde (MDA), and serum IgE levels in group C and D as compared to group B. Antioxidant treatment resulted in a significant decrease of serum Hsp70 level as compared to group B, while dexamethasone treatment resulted in a significant increase of serum Hsp70 level as compared to group B. This study suggests that it is likely that a combination of antioxidant vitamins may be effective in the treatment of asthma, considering their reported effects on lowering MDA, IL-4, and IgE levels, and the similar beneficial effects of dexamethasone in addition to increasing the expression of Hsp70 in the studied model of bronchial asthma.

Keywords: oxidative stress, heat shock protein 70, interleukin-4, immunoglobulin E, malondialdehyde, reduced glutathione, rats

Introduction

Bronchial asthma (BA) is an inflammatory disorder in which genetic and environmental factors play an important role.¹ It is a chronic inflammatory airway disease characterized by an intense eosinophilic inflammatory infiltrate on bronchial mucous membranes.² There is also infiltration of the airways with T-cells, mast cells, basophils and macrophages.³ Airway smooth muscle hypertrophy and hyperplasia are characteristics of asthma that lead to thickening of the airway wall and obstruction of airflow.⁴

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Oxygen radicals are produced by inflammatory cells in the airways and/or inhaled directly from environmental air.⁵ Oxidative stress has important implications on the pathogenesis of chronic obstructive airway diseases. These include oxidative inactivation of antiproteases and surfactants, mucous hypersecretion, membrane lipid peroxidation, mitochondrial respiration, alveolar epithelial injury, remodeling of extracellular matrix, and apoptosis.⁶ The mechanism by which oxygen radicals cause asthma pathology is through the oxidation or nitration of proteins, lipids, or DNA, to cause dysfunction in these molecules. In addition, the normal physiological antioxidant system is impaired in asthma, possibly because of inflammation. Thus, the imbalance between the oxidant and antioxidant system, called oxidative stress, is critical in asthma pathogenesis.⁵ It is believed that oxidative stress not only has important consequences for the pathogenesis of asthma, but also for the severity and treatment of this disease. It is likely, therefore, that antioxidants may be effective in the treatment of asthma.⁷ Moreover, epidemiological studies suggest that higher intakes of dietary vitamin C may be associated with a reduced risk of asthma.⁸ Increased dietary vitamin E intake is also associated with a reduced incidence of asthma, and combinations of antioxidant supplements which include vitamin E are effective in reducing ozone-induced bronchoconstriction.⁹

The heat shock proteins (Hsps) are highly conserved proteins inducible in response to a wide variety of stresses.¹⁰ Hsps are involved in various biological functions including intracellular chaperones of naïve, aberrantly folded, or mutated proteins; cytokines of signal transduction cascades involved in inflammatory response; and cytoprotective agents.¹¹ In addition, Hsps are also involved in transport of proteins and peptides through cellular compartments.¹² This suggests that Hsps may modulate immune and inflammatory responses and may be involved in the pathogenesis, and/or be markers for risk and prognosis, of certain diseases including asthma.¹³

Heat shock protein 70 (Hsp70) is recognized as having a role in chaperoning antigenic peptides and in facilitating class II peptide assembly.¹⁴ Hsp70 has been reported to block apoptosis by binding apoptosis protease activating factor-1 (Apaf-1), thereby preventing constitution of the apoptosome, the Apaf-1/cytochrome c/caspase-9 activation complex.¹⁵

Interleukin-4 (IL-4) is implicated in the pathogenesis of asthma and airway eosinophilia. It is also known for its capacity to induce mucin production and secretion.¹⁶

Activated Th2 cells, eosinophils and basophils may produce IL-4. This cytokine has a unique role in directing in immunoglobulin isotype switching towards the synthesis of immunoglobulin E (IgE). Moreover, IL-4 has a critical role in

the upregulation of IgE synthesis.¹⁷ IL-4 mediates important proinflammatory functions in asthma in induction of the IgE isotype switch, promotion of eosinophil transmigration across endothelium, mucus secretion, and differentiation of T-helper type 2 lymphocytes leading to cytokine release.¹⁸

IgE molecules play a crucial role in allergic respiratory diseases.¹⁹ An increased IgE production is the strongest predisposing factor for the development of asthma. IgE is a key component of asthma pathophysiology.²⁰ The quantity of antigen-specific IgE is thought to affect the intensity of the allergic reaction.²¹

Corticosteroids are known to exert their effects on the airway vasculature through both genomic and nongenomic mechanisms.²² Systemic treatment with glucocorticoids has been reported to inhibit smooth muscle hypercontraction which may account partially for their beneficial effects in the treatment of asthma.²³ In our study, we hypothesized that if antioxidant vitamin supplements used before an asthmatic attack could provide more or less similar effects on serum reduced glutathione (GSH), IgE, IL-4, and Hsp70 as dexamethasone, then we might recommend using them in combination with reduced doses of dexamethasone in bronchial asthma patients, thus reducing the exposure to the numerous side effects of corticosteroids. Hence, the present study was conducted to find out the effect of dexamethasone and some antioxidant vitamins on the pathogenesis of BA, and to demonstrate possible physiological benefits of using both, through studying their effects on serum levels of GSH, IL-4, IgE, and Hsp70 in an experimental model of BA in rats.

Material and methods

The study was conducted on 60 adult male albino rats weighing 150–200 g. They were allowed a chow diet and water ad libitum. All experiments were performed in accordance with national animal care guidelines and were pre-approved by the faculty ethics committee. Rats were randomly divided into 4 groups (15 for each group): including normal control group (group A); induced asthma model group where rats were sensitized by ovalbumin and challenged with antigen aerosol thus bronchial asthma was produced (group B); induced asthma model group treated with antioxidant vitamins (vitamin E and C) (group C); and lastly induced asthma model group treated with dexamethasone (group D).

Immunization and exposure of rats in groups B, C and D

On the first day of the experiment (day 0) and then again on day 12, the rats in group B, C and D were actively immunized

by intraperitoneal injection of 50 µg of ovalbumin (OVA) grade V (Sigma-Aldrich, St. Louis, MO), adsorbed to 1 mg of aluminum hydroxide (Sigma-Aldrich) in 0.9% sterile saline. On day 23, rats were exposed to an aerosol of OVA in 0.9% sterile saline for 30 min; this was repeated three times at 1-hour intervals, and then every second day thereafter, for a total of 8 days.²⁴ Signs of bronchospasm were assessed by the presence of whistling wheeze, dyspnea, acrocyanosis of paws, limbs, ears and tails, by tachypnea noted on rats, and sometimes, convulsions. The aerosol was generated with an ultrasonic nebulizer (Sunrise, Devilbiss Medical, Nantes, France). According to the specifications supplied by the manufacturer, the output of the nebulizer is 0.5 mL/min, and the particles produced have a mean diameter of 3.5 µm. The concentration of OVA in the nebulizer was 10 mg/mL.²⁴

The same procedures of immunization by intraperitoneal injection and exposure to aerosol were applied to the control group using 0.9% saline. Twenty-four hours after the last aerosol exposure, the rats in group A and B were anesthetized by intraperitoneal injection of pentobarbital 80 mg/kg body weight (Tokyo Kasei, Tokyo, Japan) and sacrificed, following which blood and lung samples were collected.

Group C received diet mixed with 400 mg/kg/day of vitamin E (α -tocopherol) in their food, and vitamin C (calcium L-ascorbate dihydrate) (500 mg/100 mL of drinking water) using nasogastric tube.^{25,26} The rats in group C were treated with vitamins E and C from day 0 of the experiment and continued for twelve weeks. Drugs were obtained from the Sigma and Fluka Company (Sigma Aldrich, Prague, Czech Republic).

Rats in group D were immunized and exposed to OVA aerosol challenge as previously explained above, after which the OVA aerosol challenges were repeated twice weekly for 3 months. The rats in this group were intraperitoneally injected with dexamethasone (Changzhou Second Pharmaceuticals Co., Changzhou, China) (1 mg/kg in 100 µL of sterile PBS). The first dose of dexamethasone was administered 6 hours before the first OVA challenge (day 23) and dosage was then continued daily for duration of the 12-week period of twice weekly OVA aerosol challenges.²⁷ Rats in group C and D were anesthetized by intraperitoneal injection of phenobarbital 80 mg/kg body weight, and sacrificed 24 hours after the last doses of the antioxidant vitamins and dexamethasone respectively, at which time blood and lung samples were collected.

Venous blood samples were collected from all groups in dry test tubes without additives and left to clot, then the blood was centrifuged at 3000 rpm for 15 minutes at 4°C to separate the serum and stored at -80°C until used for estimation of Hsp70,

IL-4, IgE, and GSH. The remaining erythrocytes were washed three times in cold saline, then hemolyzed using ice cold distilled water at a rate of 1–4 volumes. Cell membrane was removed by centrifugation and the hemolysates were frozen in aliquots at -80°C for analysis of MDA.

Lung histology

The lungs were resected, fixed in 10% phosphate-buffered formalin, embedded into paraffin, sectioned, stained with hematoxylin-eosin solution, and examined by light microscopy for histologic changes.

Estimation of heat shock protein 70 (Hsp70), interleukin-4 (IL-4) and immunoglobulin-E (IgE) immunoassays^{28–30}

These assays employ the quantitative sandwich enzyme immunoassay technique: Assay Designs Stressgen High Sensitivity EIA Kit for HSP70, the IL-4 immunoassay kit was purchased from Quantikine, R and D Systems, USA and the high sensitive ELISA kit for IgE was purchased from BioVendor, Laboratorní Medicína, Czech Republic.

GSH contents were determined via the method of Miwa et al.³¹ GSH determination is based on the development of a yellow color when 5,5'-dithio (2-nitro benzoic acid) (DTNB) is added to compounds containing sulfhydryl groups. The values are expressed as mg/100 mL.³²

MDA was used as a biochemical marker for lipid peroxidation, and was measured by the method described by Ohkawa et al.³³ using thiobarbituric acid (TBA).

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 15) software. Results were expressed as means \pm SD (mean \pm SD). One-way ANOVA (F) test was done to make the comparison between different groups. $P < 0.05$ was considered statistically significant.

Results

Serum reduced glutathione (GSH) in mg/100 mL

The results of the present study showed a significant decrease of serum GSH levels in groups B, C and D as compared to the control group A, (27.39 ± 1.22 , 30.76 ± 1.44 , and $31.36 \pm 1.44^*$ vs 36.95 ± 4.18 mg/100 mL, $P < 0.05$). However, there was a significant increase of serum GSH levels in groups C and D as compared to group B, (30.76 ± 1.44 , and $31.36 \pm 1.44^*$ vs 27.39 ± 1.22 mg/100 mL, $P < 0.05$) ($F = 40.768$) (Table 1, Figure 1).

Table I Serum reduced glutathione (GSH) levels in mg/100 mL

Parameter	Groups	Min.	Max.	Mean	S.D.	F	P
GSH (mg/100 mL)	Group A	33.30	49.60	36.95 ^a	4.18	40.768	0.0002*
	Group B	26.03	29.50	27.39 ^b	1.22		
	Group C	28.80	33.80	30.76 ^c	1.44		
	Group D	28.60	35.30	31.36 ^c	1.44		

Notes: Serum GSH levels in mg/100 mL among the studied groups expressed as mean \pm SD.

*significant at $P < 0.05$ compared to groups A, B, C and D. The same small letters (c, c) indicate there was no significant difference, while the different letters (a, b, c) indicate there was a significant difference.

Serum interleukin-4 (IL-4) in pg/mL

There was a significant increase of serum IL-4 in groups B and C as compared to group A (50.91 ± 1.13 and $48.14 \pm 0.47^*$ vs 46.10 ± 0.20 pg/mL, $P < 0.05$). Antioxidant treatment and dexamethasone treatment resulted in a significant decrease of serum IL-4 levels in groups C and D as compared to group B (48.14 ± 0.47 and $46.20 \pm 0.17^*$ vs 50.91 ± 1.13 pg/mL, $P < 0.05$) ($F = 193.204$) (Table 2, Figure 2).

Red blood cell hemolysates malondialdehyde (MDA) in mmol/g hemoglobin

A significant increase of MDA levels was reported in groups B and C as compared to group A (2.97 ± 0.24 and $2.19 \pm 0.05^*$ vs 2.06 ± 0.03 mmol/g hemoglobin, $P < 0.05$). MDA level was significantly decreased in groups C and D as compared to group B (2.19 ± 0.05 and $2.07 \pm 0.02^*$ vs 2.97 ± 0.24 mmol/g hemoglobin, $P < 0.05$) ($F = 180.094$) (Table 3, Figure 3).

Serum immunoglobulin E (IgE) levels in μ g/mL

As shown in Table 4, there was a significant increase of serum IgE levels in groups B and C as compared to the control group (2.36 ± 0.11 and $1.76 \pm 0.17^*$ vs 1.17 ± 0.01 μ g/mL, $P < 0.05$).

Group C and group D rats showed a significant decrease of serum IgE levels as compared to group B (1.76 ± 0.17 and $1.19 \pm 0.02^*$ vs 2.36 ± 0.11 μ g/mL, $P < 0.05$) ($F = 436.383$) (Table 4, Figure 4).

Serum heat shock protein 70 (Hsp70) in ng/mL

Our results showed a significant increase of serum Hsp70 in groups B and D as compared to group A (12.63 ± 0.13 and $14.52 \pm 0.15^*$ vs 10.68 ± 0.15 ng/mL, $P < 0.05$). Antioxidant treatment (group C) resulted in a significant decrease of serum Hsp70 level as compared to group B ($10.67 \pm 0.11^*$ vs 12.63 ± 0.13 ng/mL, $P < 0.05$), while

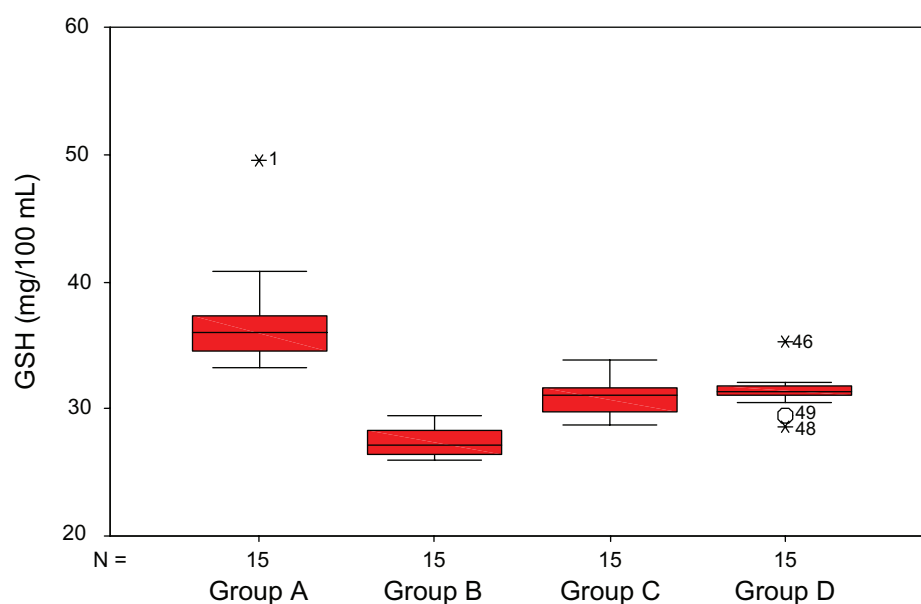


Figure 1 Serum reduced glutathione (GSH) in mg/100 mL. Serum GSH levels in mg/100 mL among the studied groups. Serum GSH levels were significantly decreased in groups B, C, and D as compared to group A. While they were significantly increased in induced bronchial asthma groups treated with antioxidant and dexamethasone (groups C and D) as compared to the induced bronchial asthma group (group B).

Table 2 Serum interleukin-4 (IL-4) in pg/mL

Parameter	Groups	Min.	Max.	Mean	S.D.	F	P
IL-4 (pg/mL)	Group A	45.80	46.40	46.10 ^a	0.20	193.204	0.0001*
	Group B	49.70	53.50	50.91 ^b	1.13		
	Group C	47.50	48.90	48.14 ^c	0.47		
	Group D	46.00	46.50	46.20 ^a	0.17		

Notes: Serum IL-4 in pg/mL among the studied groups expressed as mean \pm SD.

*significant at $P < 0.05$ compared to groups A, B, C and D. The same small letters (a, a) indicate there was no significant difference, while different letters (a, b, c) indicate there was a significant difference.

dexamethasone treatment (group D) resulted in a significant increase of serum Hsp70 level as compared to group B ($14.52 \pm 0.15^*$ vs 12.63 ± 0.13 ng/mL, $P < 0.05$) ($F = 2721.89$) (Table 5, Figure 5).

Lung histology

The results of the present work revealed the histological patterns shown in Figures 6 (a, b, c, d and e). The airways in induced BA (group B) showed the characteristic occlusion by tenacious plugs of exudates and mucus. There was also thickening of the reticular layer beneath the epithelial basal lamina, and increased inflammatory infiltrate comprising 'activated' lymphocytes and eosinophils. The control group showed normal bronchial wall, and scanty mononuclear cells were seen in the lamina propria (Figures 6 a, b, c). Antioxidant treatment (group C) showed less mucus secretion and inflammation than group B (Figure 6 d). Group D treated with dexamethasone revealed a total clearance of secretions, with fewer eosinophils (Figure 6 e).

Discussion

In the pathogenesis of bronchial asthma, the sensitization and allergization of the mast cell lead to its degranulation, with the subsequent release of mediators which result in bronchoconstriction and the production of viscous mucus.³⁴

The results of the present study showed a significant decrease of serum GSH levels in groups B, C and D as compared to the control group A. This may be explained by allergens inducing chronic airway inflammation through the generation of reactive oxygen species, causing oxidative stress. Mean blood levels of GSH were reported to be significantly lower in bronchial asthma cases, which can be explained by oxidative stress.³⁵

Another study investigating daily micronutrient/antioxidant intake and asthma severity revealed reduction of platelet GSH-peroxidase activity in the most severe cases, suggesting that these patients have a diminished capacity to restore part of the antioxidant defenses.³⁶

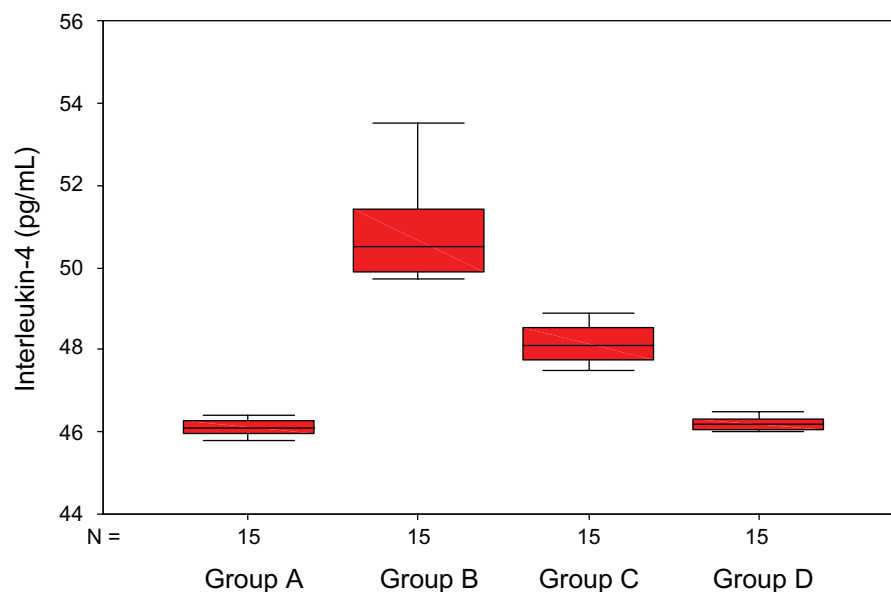


Figure 2 Serum interleukin-4 (IL-4) in pg/mL. Serum IL-4 levels were significantly increased in groups B and C as compared to group A. Antioxidant and dexamethasone treated groups (groups C and D) showed a significant decrease in 9 serum IL-4 levels as compared to induced bronchial asthma group (group B).

Table 3 Red blood cell hemolysate malondialdehyde (MDA) levels in mmol/g hemoglobin

Parameter	Groups	Min.	Max.	Mean	SD	F	P
MDA (mmol/g hemoglobin)	Group A	2.02	2.09	2.06 ^a	0.03	180.094	0.0001*
	Group B	2.09	3.08	2.97 ^b	0.24		
	Group C	2.15	2.27	2.19 ^c	0.05		
	Group D	2.05	2.09	2.07 ^a	0.02		

Notes: Red blood cell hemolysate MDA levels in mmol/g hemoglobin expressed as mean \pm SD.

*significant at $P < 0.5$ compared to groups A, B, C and D. The same small letters (a, a) indicate there was no significant difference, while the different letters (a, b, c) indicate there was a significant difference.

Our results also showed a significant increase of serum GSH levels in groups C and D as compared to group B. This is also in accord with previous research that demonstrated a role for vitamin C and E in maintaining glutathione in its reduced form, and their ability to compensate for GSH depletion. Both of these findings support their preventive or therapeutic use in combating age- and pathology-associated declines in GSH.³⁷

Recent research has demonstrated significant alterations of GSH homeostasis in children with severe refractory asthma, characterized by decreased GSH, increased GSSG, and greater oxidation as measured by the GSH redox potential.³⁸ Although previous studies have provided some support for the role of dietary antioxidants in allergic airway disease, the results of antioxidant supplementation studies have been largely disappointing. However, these previous studies focused on clinical outcomes such as pulmonary function and did not take into account the effects of

antioxidant therapy on extracellular antioxidant balance or intracellular signaling.³⁹

Administration of exogenous corticosteroids has been reported to accelerate the late gestational rise in fetal rat lung antioxidant enzyme activity, however the effect of dosing intervals on these responses remains uncertain. What has been demonstrated is the positive effect of a single dose of betamethasone on lung antioxidant enzyme activity.⁴⁰

As regards IL-4 levels, there was a significant increase of serum IL-4 in groups B and C as compared to group A. An essential biological activity of IL-4 in the development of allergic inflammation is to drive the differentiation of naïve helper T-cells (Th0 cells) into Th2 cells, which secrete IL-4.^{41,42} Accumulating evidence indicates that T helper cell-derived cytokines, such as IL-4, play a critical role in orchestrating and amplifying allergic inflammation in asthma.⁴³ In our study antioxidant and dexamethasone treatment resulted in a significant decrease of serum IL-4 levels

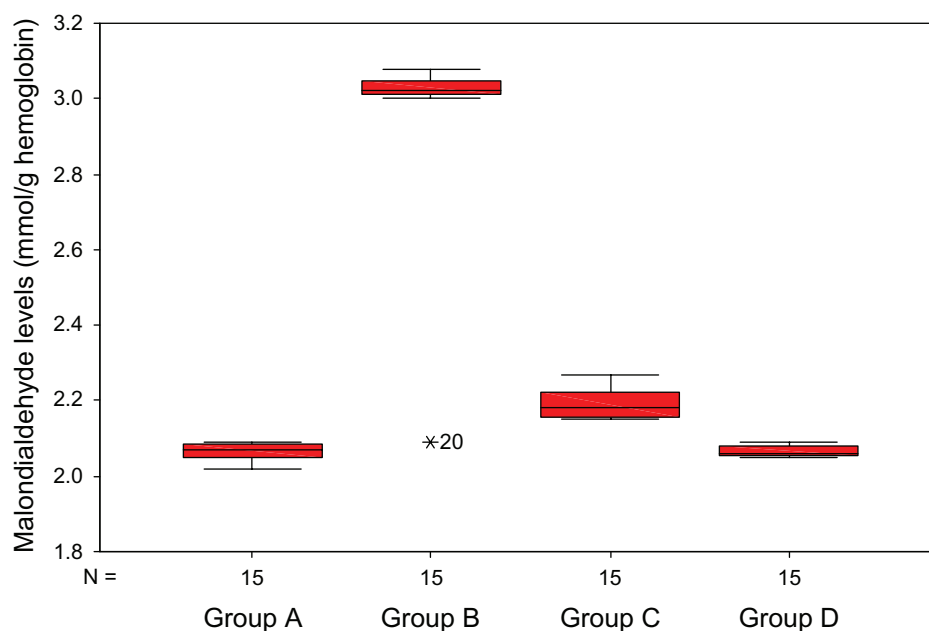


Figure 3 Red cell hemolysates malondialdehyde levels (MDA) in mmol/g hemoglobin. Red cell hemolysates MDA levels showed a significant increase in groups B and C when compared to group A. There was a significant decrease in their levels in antioxidant and dexamethasone treated groups (groups C and D) compared to the induced bronchial asthma group (group B).

Table 4 Serum immunoglobulin E (IgE) levels in $\mu\text{g/mL}$

Parameter	Groups	Min.	Max.	Mean	SD	F	P
IgE levels ($\mu\text{g/mL}$)	Group A	1.15	1.19	1.17 ^a	0.01	436.383	0.00001*
	Group B	2.23	2.53	2.36 ^b	0.11		
	Group C	1.45	2.00	1.76 ^c	0.17		
	Group D	1.17	1.22	1.19 ^a	0.02		

Notes: Serum IgE levels $\mu\text{g/mL}$ expressed as mean \pm SD.

*significant at $P < 0.05$ compared to groups A, B, C and D. The same small letters (a, a) indicate there was no significant difference, while different letters (a, b, c) indicate there was a significant difference.

in groups C and D as compared to group B, which may be beneficial in decreasing allergic inflammation in BA.

Vitamin C has been implicated in immune response. In fact, *in vivo* administration of vitamin C modulates T cell proliferation and cytokine secretion.⁴⁴ IL-4 plays a pivotal role in the development of allergic inflammation via the induction of IgE isotype switching, an increase of IgE receptor expression, promoting Th2 cell differentiation, and stimulating several genes involved in atopic disorders.⁴⁵ Previous data has shown that vitamin E suppresses IL-4 protein levels in human peripheral blood T- cells in a dose-dependent manner. Such data provide molecular evidence supporting the beneficial effect of dietary vitamin E on atopic disorders.⁴⁶

However, other research has demonstrated that supplementation of sufficiently fed non-stressed, young adult mice with vitamins C had no effect on immune function. They speculated that using this model in aged, physiologically,

or nutritionally stressed mice may provide outcomes more similar to those in sensitive human populations.⁴⁷

A significant increase of MDA levels was reported in groups B and C as compared to group A. MDA is considered a marker of end- stage lipid peroxidation. Plasma levels of MDA were reported to be elevated in bronchial asthma patients which could be explained by the imbalance between increased production of free oxygen radicals and decreased antioxidative defense. This imbalance could be considered as the important triggering factor, as well as the factor responsible for the maintenance of chronic inflammation.⁴⁸ According to our findings, MDA level was significantly decreased in groups C and D as compared to group B, which could explain the protective role of antioxidants and dexamethasone in BA. It has been recently demonstrated that vitamin C treatment in pregnancy significantly reduced lipid peroxidation and MDA production during pregnancy.⁴⁹ On the other hand, plasma MDA levels showed no differences among experimental hyperthyroid animals and

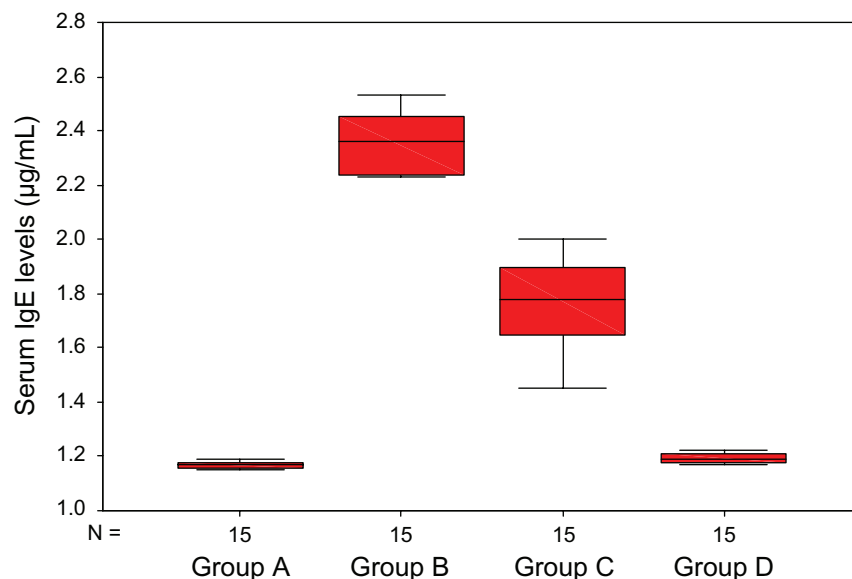


Figure 4 Serum immunoglobulin E (IgE) levels in $\mu\text{g/mL}$. Serum IgE levels were significantly increased in groups B and C as compared to group A. There was a significant decrease in IgE in the antioxidant and dexamethasone treated groups (groups C and D) compared to induced bronchial asthma group (group B).

Table 5 Serum heat shock protein 70 (Hsp70) in ng/mL

Parameter	Groups	Min.	Max.	Mean	S.D.	F	P
Hsp70 (ng/mL)	Group A	10.50	11.02	10.68 ^a	0.15	2721.89	0.0001*
	Group B	12.40	12.80	12.63 ^b	0.13		
	Group C	10.40	10.80	10.67 ^a	0.11		
	Group D	14.20	14.80	14.52 ^c	0.15		

Notes: Serum Hsp70 in ng/mL among the studied groups expressed as mean \pm SD.

*significant at $P < 0.05$ compared to groups A, B, C and D. The same small letters (a, a) indicate there was no significant difference, while different letters (a, b, c) indicate there was a significant difference.

those supplemented with vitamin C and E. Having said that, MDA levels measured in non-HDL fraction were significantly lower in the vitamin-supplemented groups, which indicates that vitamin E, as well as vitamin C, supplementation protect these lipoproteins from oxidation.⁵⁰

Hanta et al 2003 used mild asthmatic patients divided into inhaled steroids and no inhaled steroid user; this study found no significant differences in the measurements of plasma MDA level between the mentioned subgroups. The previous study also suggested that oxidant-antioxidant balance is not significantly affected in mild asthmatics or measurement of plasma level of MDA is not sensitive to the oxidant-antioxidant balance in mild asthmatics.⁵¹

Previous research done to study of the effect of dexamethasone on small bowel and kidney oxidative stress found that the mean serum MDA level showed a significant decrease among the dexamethasone-treated group as

compared to the untreated one.⁵² Similarly, lipid peroxidation, as shown by serum and cerebrospinal MDA, was increased in multiple sclerosis patients and reduced with corticosteroid therapy.⁵³

The results of the present study showed a significant increase in serum IgE levels in groups B and C as compared to the group A. Previous studies have reported that the highest levels of IgE and eosinophil infiltration were achieved after systemic sensitization with allergen (plus adjuvant) followed by repeated airway challenge.⁵⁴ Elevated levels of IgE are associated with bronchial asthma. Activation of antigen-specific T helper (Th) 2 cells in the lung, with the subsequent release of IL-4 and IL-5, is believed to play an important role in the pathogenesis of this disease.⁵⁵

Group C and group D rats showed a significant decrease of serum IgE levels as compared to group B. Investigating the relationship between dietary vitamin E intake and serum IgE

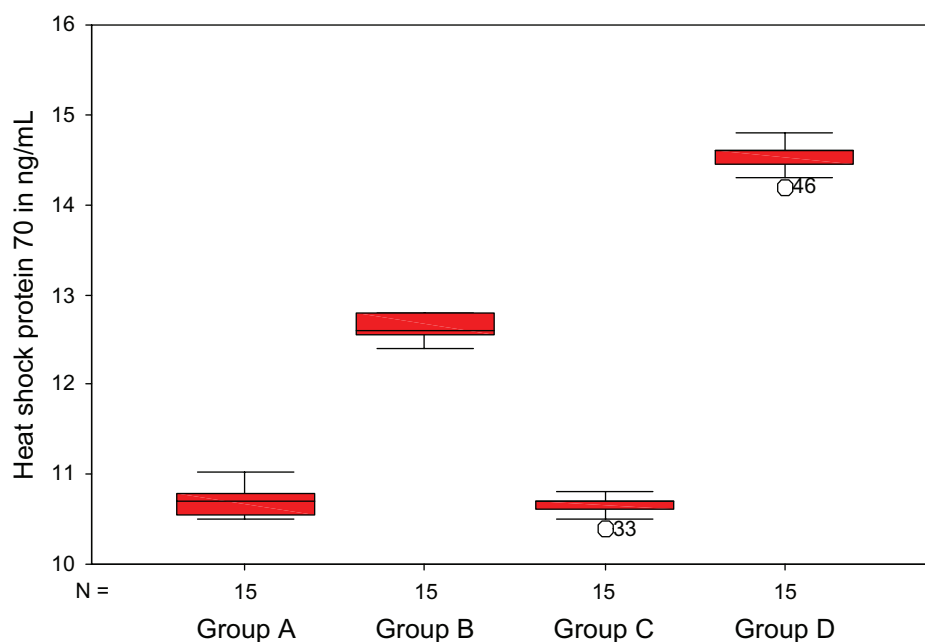


Figure 5 Serum heat shock protein 70 (Hsp70) in ng/mL. Serum Hsp70 levels showed a significant increase in groups B and D compared to group A. Induced bronchial asthma group treated with antioxidant (group C) showed a significant decrease in serum HSP70 as compared to the induced bronchial asthma group (group B), while induced bronchial asthma group treated with dexamethasone (group D) showed a significant increase in serum HSP70 levels as compared to the induced bronchial asthma group (group B).

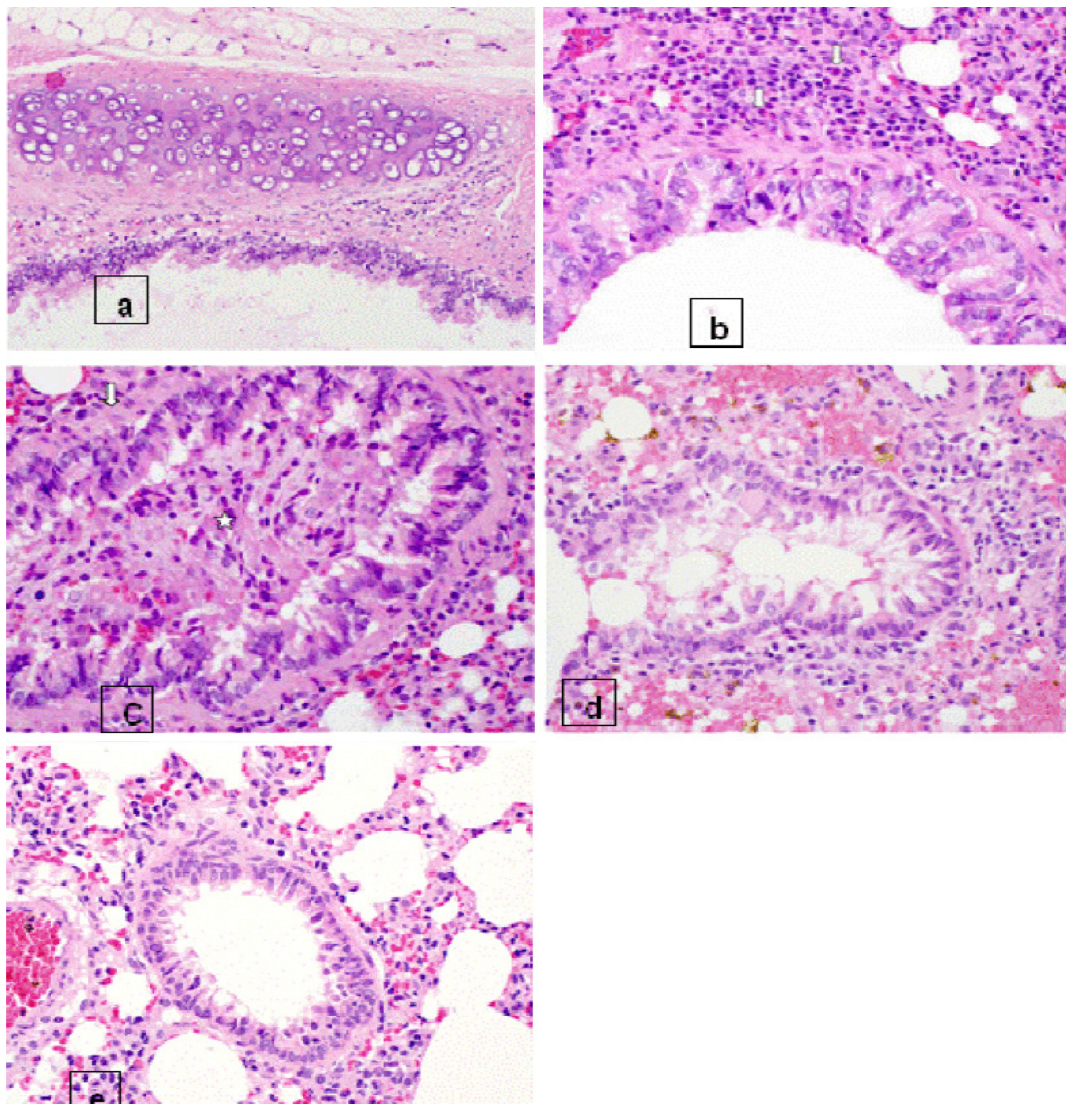


Figure 6 Histological pattern of bronchial wall in control and induced bronchial asthma stained with H&E. **a)** Normal bronchial wall in control group. Scanty mononuclear cells are seen in lamina propria (2 \times); **b)** Induced bronchial asthma group. The lamina propria shows moderate infiltration by mononuclear cells mixed with several eosinophils (arrow) (40 \times); **c)** Induced bronchial asthma group. The airways in asthma are occluded by tenacious plugs of exudates and mucus (star). There is thickening of the reticular layer beneath the epithelial basal lamina (arrow). There is increased inflammatory infiltrate comprising 'activated' lymphocytes and eosinophils (40 \times); **d)** Induced bronchial asthma group treated with antioxidant. Bronchiolar wall shows fewer amounts of mucous secretion and inflammation than bronchial asthma group (40 \times); **e)** Induced bronchial asthma group treated with corticosteroids. The bronchial lumen show total clearance of secretion. A few mononuclear cells are seen around the bronchiole with occasional eosinophils (40 \times).

concentrations and atopy, higher concentrations of vitamin E intake were associated with lower serum IgE concentrations, and a lower frequency of allergen sensitization.⁵⁶ These findings may explain the beneficial effect of dietary vitamin E on the incidence of asthma.

Previous research demonstrates that piglets which were not supplemented with vitamin C presented an increase in serum IgE, whereas vitamin C supplementation attenuated this, by increasing interferon- γ , whilst decreasing IL-4, in the cultured primary splenocytes, which suppresses the immune response. These results suggest that a mega-dose of vitamin C can be used to prevent soybean allergies.⁵⁷

Our results showed a significant increase of serum Hsp70 in group B as compared to group A. Recent research suggests that Hsp70 overexpression in asthma results from complex interactions between environmental exposures and genetic background, rather than from specific genetic variations in *hsp70* genes.⁵⁸ Hsp70 are recognized to have a role in chaperoning antigenic peptides and in facilitating class II peptide assembly. Hsp70 over-expression implies a potential role for these proteins in antigen processing and/or presentation, resulting in an increased activity of antigen presenting cells.⁵⁹ Group C showed a significant decrease of serum Hsp70 level as compared to group B, while group D showed

a significant increase of serum Hsp70 level as compared to group B. This may be explained by an increase in other antioxidant protective mechanisms which recommends further studies. Oxidative stress induces adaptations in the expression of protective enzymes and heat shock proteins (Hsps) in a variety of tissues. The possibility was also examined that supplementation of subjects with vitamin C influences the ability of lymphocytes to express protective enzymes and Hsps following exposure to an exogenous oxidant. Hsp70 content of lymphocytes from supplemented subjects did not increase significantly in response to hydrogen peroxide. It was concluded that adaptive responses to oxidants are attenuated in vitamin C-supplemented subjects, but it is also possible that this may reflect an increased baseline expression of potential protective systems against oxidative stress as superoxide dismutase or catalase.⁶⁰ Dexamethasone-related Hsp70 induction might be explained by a possible cooperation of heat shock factor and glucocorticoid receptor in Hsp70 gene expression.⁶¹ Finally, the histological results of our present study showed significant improvement with both antioxidant and dexamethasone treatment, which goes hand in hand with the biochemical results.

Conclusion

This study suggests that it is likely that a combination of antioxidant vitamins may be effective in the treatment of asthma, taking into consideration their reported effects on increasing serum GSH levels and lowering serum MDA, IL-4, and IgE levels, and the similar beneficial effects of dexamethasone in addition to increasing the expression of Hsp70 in the studied model of bronchial asthma.

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References

- Gupta V, Sarin BC, Changotra H, Sehajpal PK. Association of G-308A TNF- α polymorphism with bronchial asthma in a north Indian population. *J Asthma*. 2005;42(10):839–841.
- Carneiro ER, Carneiro CRW, De Castro MA, Yamamura Y, Silveira VL. Effect of electroacupuncture on bronchial asthma induced by ovalbumin in rats. *J Alternative Compl Med*. 2005;11(1):127–134.
- Hamid Q, Tulic MK, Liu MC, Moqbel R. Inflammatory cells in asthma: mechanisms and implications for therapy. *J Allergy Clin Immunol*. 2003;111(1):S5–S12.
- Salinhone S, Ba M, Hanson L, Martin JL, Halayko AJ, Gerthoffer WT. Overexpression of human Hsp27 inhibits serum-induced proliferation in airway smooth muscle myocytes and confers resistance to hydrogen peroxide cytotoxicity. *Am J Physiol Lung Cell Mol Physiol*. 2007;293:L1194–L1207.
- Fujisawa T. Role of oxygen radicals on bronchial asthma. *Current Drug Targets-Inflammation and Allergy*. 2005;4(4):505–509.
- Rahman I. The role of oxidative stress in the pathogenesis of COPD: implications for therapy. *Treat Respir Med*. 2005;4(3):175–200.
- Kirkham P, Rahman I. Oxidative stress in asthma and COPD: antioxidants as a therapeutic strategy. *Pharmacol Ther*. 2006;111(2):476–494.
- Fogarty A, Lewis SA, Scrivener SL, et al. Oral magnesium and vitamin C supplements in asthma: a parallel group randomized placebo-controlled trial. *Clin Exp Allergy*. 2003;33(10):1355–1359.
- Pearson PJ, Lewis SA, Britton J, Fogarty A. Vitamin E supplements in asthma: a parallel group randomized placebo-controlled trial. *Thorax*. 2005;60(2):171–172.
- Michaud S, Marin R, Tanguay RM. Regulation of heat shock gene induction and expression during Drosophila development. *Cell Mol Life Sci*. 1997;53:104–113.
- Asea A, Kraeft SK, Kurt Jones E, et al. Hsp70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med*. 2000;6:435–442.
- Singh-Jasuja H, Hilf N, Arnold-Schild D, Schild H. The role of heat shock proteins and their receptors in activation of the immune system. *Biol Chem*. 2001;382:629–636.
- Xiao C, Wu T, Ren A, et al. Basal and inducible levels of Hsp70 in patients with acute heat-induced illness induced during training. *Cell Stress Chaperones*. 2003;8:86–92.
- Bertorelli G, Bocchino V, Zhuo X, et al. Heat shock protein 70 upregulation is related to HLA-DR expression in bronchial asthma. Effects of inhaled glucocorticoids. *Clin Exp allergy*. 1998;28(5):551–560.
- Ravagnan L, Gurbuxani S, Susin SA, et al. Heat shock protein 70 antagonizes apoptosis-inducing factor. *Nat Cell Biol*. 2001;3(9):839–843.
- Finkelman FD, Katona IM, Urban JFJ, Snapper CM, Ohara J, Paul WE. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. *Proc Natl Acad Sci U S A*. 1986;83(24):9675–9678.
- Marsh DG, Neely JD, Beazeale DR, et al. Linkage analysis of IL-4 and other chromosome 5p31.1 markers and total serum IgE concentrations. *Science*. 1994;264:1152–1155.
- Steinke JW, Borish L. Th2 cytokines and asthma interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment interleukin-4 receptor antagonist. *Resp Res*. 2001;2(2):66–70.
- D'Amato G. Role of anti-IgE monoclonal antibody (omalizumab) in the treatment of bronchial asthma and allergic respiratory diseases. *Eur J Pharmacol*. 2006;533(1–3):302–307.
- Skiepkowski R, Zietkowski Z, Tomasiak-Lozowska MM, Bodzenta-Lukaszyk A. Anti-IgE therapy in persistent allergic asthma. *Przegl Lek*. 2009;66(3):141–144.
- Liang Y, Ganley-Leal LM. A simple method for measuring human cell-bound IgE levels in whole blood. *J Immunol Methods*. 2009 (ahead of print).
- Horvath G, Wanner A. Inhaled corticosteroids: effects on the airway vasculature in bronchial asthma. *Respir J*. 2006;27:172–187.
- Chiba Y, Goto K, Hirahara M, Sakai H, Misawa M. Glucocorticoids ameliorate antigen-induced bronchial smooth muscle hyperresponsiveness by inhibiting upregulation of RhoA in rats. *J Pharmacol Sci*. 2008;106(4):615–625.
- Akimoto T, Numata F, Tamura M, et al. Abrogation of Bronchial Eosinophilic Inflammation and Airway Hyperreactivity in Signal Transducers and Activators of Transcription (STAT) 6-deficient Mice. *J Exp Med*. 1998;187(9):1537–1542.
- Suchankova J, Vopravsalova M, Kottova M, Vsemecny V, Visnovsky P. Effects of oral alpha-tocopherol on lung response in rat model of allergic asthma. *Respirology*. 2006;11(4):414–421.
- Nishikawa Y, Tatsumi K, Matsuura T, Yamamoto A, Nadamoto T, Urabe K. Effects of vitamin C on high blood pressure induced by salt in spontaneously hypertensive rats. *J Nutr Sci Vitaminol*. 2003;49(5):301–309.
- Miller M, Cho JY, McElwain K, et al. Corticosteroids prevent myofibroblast accumulation and airway remodeling in mice. *Am J Physiol Lung Cell Mol Physiol*. 2005;290:L162–L169.

28. Gray CC, Amrani M, Yacoub MH. Heat stress proteins and myocardial protection: experimental model or potential clinical tool? *Int J Biochem Cell Biol.* 1999;31(5):559–573.
29. Ishikawa S, Hashida S, Hashinaka K, Ishikawa E. Ultrasensitive enzyme immunoassay of antibody IgG to HIV-1 reverse transcriptase by immune complex transfer with detergents. *Analytical letters.* 2000;33(11):2183–2196.
30. Warbrick EV, Dearman R J, Kimber I. Induced changes in total serum IgE concentration in the Brown Norway rat: Potential for identification of chemical respiratory allergens. *J Appl Toxicol.* 2002;22:1–11.
31. Miwa S, Luzzatto L, Rosa R, et al. International committee for standardization in haematology. Recommended methods for an additional red cell enzyme (pyrimidine 5'-nucleotidase) assay and the determination of red cell adenosine 5'-triphosphate, 2,3-diphosphoglycerate and reduced glutathione. *Clin Lab Haematol.* 1989;11:131–136.
32. Griffith W. Glutathione and glutathione disulfide. In: Bergmeyer H U, editor. *Methods of enzymatic analysis.* New York: Weinheim and Academic Press; 1985:521–529.
33. Ohkawa H, Ohnishi N, Yagi K. Assay for lipid peroxides for animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351–358.
34. Gotz M, Sanz K. Pollinosis and bronchial asthma: pathogenesis, immunology, clinical aspects. *Pediatr Padol.* 1990;25(4):239–247.
35. Suresh R, Awasthi S, Mahdi AA, Patel DK, Singh VK, Rita M. Assessment of association of exposure to polycyclic aromatic hydrocarbons with bronchial asthma and oxidative stress in children: A case control study. *Indian J Occup Environ Med.* 2009;13(1):33–37.
36. Picado C, Deulofeu R, Lleonart R, et al. Dietary micronutrients/antioxidants and their relationship with bronchial asthma severity. *Eur J allergy Clin Immunol.* 2009;56(1):43–49.
37. Shang F, Lu M, Dudek E, Reddan J, Taylor A. Vitamin C and vitamin E restore the resistance of GSH-depleted lens cells to H₂O₂. *Free Radic Biol Med.* 2003;34(5):521–530.
38. Fitzpatrick AM, Teague WG, Holguin F, Yeh M, Brown LS. Airway glutathione homeostasis is altered in children with severe asthma: Evidence for oxidant stress. *J Allergy Clin Immunol.* 2009;123(1):146–152.
39. Devereux G. Diet as a risk factor for atopy and asthma. *J Allergy Clin Immunol.* 2005;115:1109–1117.
40. Walther FJ, David-Cu R, Mehta EI, Polk DH, Jobe AH, Ikegami M. Higher lung antioxidant enzyme activity persists after single dose of corticosteroids in preterm lambs. *Am J Physiol Lung Cell Mol Physiol.* 1996;271(2):L187–L191.
41. O'Garra A, Arai N: The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends Cell Biol.* 2000;10:542–550.
42. Murphy KM, Reiner SL: The lineage decisions of helper T cells. *Nat Rev Immunol.* 2002;2:933–944.
43. Nakajima H. Role of cytokines in allergic airway inflammation. *Int Arch Allergy Immunol.* 2007;142(4):265–273.
44. Maeng HG, Lim H, Jeong YJ, et al. Vitamin C enters mouse T cells as dehydroascorbic acid in vitro and does not recapitulate in vivo vitamin C effects. *Immunobiology.* 2009;214(4):3113–3120.
45. Steinke JW, Borish L. Th2 cytokines and asthma – Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. *Resp Res.* 2001;2:66–70.
46. Li-Weber M, Giaisi M, Treiber MK, Krammer PH. Vitamin E inhibits IL-4 gene expression in peripheral blood T cells. *Eur J Immunol.* 2002;32(9):2401–2408.
47. Albers R, Bol M, Bleumink R, Willems A, Peters R. Effects of supplementation with vitamins A, C, and E, selenium, and zinc on immune function in a murine sensitization model. *Nutrition.* 2003;19(11):940–946.
48. Dworski R, Roberts LJ 2nd, Murray JJ, Morrow JD, Hartert TV, Sheller JR. Assessment of oxidant stress in allergic asthma by measurement of the major urinary metabolite of F2-isoprostane, 15-F2t-IsoP(8-iso-PGF2alpha). *Clin Exp Allergy.* 2001;31:387–390.
49. Olayakil LA, Ajao SM, Jimoh AA, Aremu I T, Soladoye AO. Effect of vitamin C on malondialdehyde (MDA) in pregnant Nigerian women. *J Basic Applied Sci.* 2008;4(2):105–108.
50. Dirican M, Tas S. Effects of vitamin E and vitamin C supplementation on plasma lipid peroxidation and on oxidation of apolipoprotein B-containing lipoproteins in experimental hyperthyroidism. *J Med Invest.* 1999;46(1/2):29–33.
51. Hanta I, Kuleci S, Canacankatan S, Kocabas A. The oxidant-antioxidant balance in mild asthmatic patients. *Lung.* 2003;181(6):347–352.
52. Ozturk H, Eken H, Ozturk H, Buyukbayram H. Effects of dexamethasone on small bowel and kidney oxidative stress and histological alterations in bile duct-ligated rats. *Pediatr Surg Int.* 2006;22(9):709–718.
53. Keles MS, Taysi S, Aksoy H, Akçay F, Sen N. Effect of corticosteroid therapy on serum and CSF malondialdehyde and antioxidant proteins in multiple sclerosis. *Canadian Journal of Neurological Science.* 2001;28(2):141–143.
54. Hamelmann E, Tateda K, Oshiba A, Gelfand EW. Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness—a murine model. *Allergy.* 1999;54(4):297–305.
55. Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J Exp Med.* 1996;183:1303–1310.
56. Fogarty A, Lewis S, Weiss S, Britton J. Dietary vitamin E, IgE concentrations, and atopy. *Lancet.* 2009;356(9241):1573–1574.
57. Sun P, Li D, Dong B, Qiao S, Ma X, Chen X. Vitamin C: an immune modulator that attenuates anaphylactic reactions to soybean glycinin hypersensitivity in a swine model. *Food Chem.* 2009;113(4):914–918.
58. Aron Y, Busson M, Polla BS, et al. Analysis of hsp70 gene polymorphism in allergic asthma. *Allergy.* 2001;54(2):1650–1670.
59. Bertorelli G, Bocchino V, Zhuo X, et al. Heat shock protein 70 upregulation is related to HLA-DR expression in bronchial asthma. Effects of inhaled glucocorticoids. *Clin Exp Allergy.* 1998;28(5):551–560.
60. Khassaf M, McArdle A, Esanu C, et al. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J Physiol.* 2003;549(Pt 2):645–652.
61. Konishi T, Nomoto M, Shimizu K, et al. Dominant role of the second heat shock element in expression of the HSP70-1 gene in rat liver after whole body hyperthermia. *J Biochem.* 1995;118:1021–1029.

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